LABORATORY

79 Laboratory Evaluation of the Autonomic System

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Definition

The autonomic nervous system (ANS) controls body functions that can proceed independent of volitional activity. The system consists of a somatic afferent pathway, a central nervous system (CNS) integrating complex (brain and spinal cord), and two distinct efferent limbs (sympathetic and parasympathetic) each made up of preganglionic and postganglionic neurones. The viscera are innervated by both sympathetic and parasympathetic fibers, and the ANS controls the activity of the cardiovascular, respiratory, gastrointestinal, and genitourinary systems, and has effects on smooth muscle, blood vessels, and sweat glands, and on the endocrine system. It provides a mechanism that influences the end organs through chemical transmitters: catecholamines at the sympathetic and acetylcholine at the parasympathetic nerve endings. Centrally, acetylcholine, monoamines, and several peptides in the hypothalamus and brainstem may modulate the system.

Technique

Skin

Sweating can be provoked by increasing the body's core temperature, indicating that the efferent sympathetic sudomotor pathway is intact. In diabetes, sweating is initially lost distally, first from the feet and then it progresses proximally.

The core temperature can be raised by 1°C over a period of 90 minutes in different ways using a heat cradle, a hot bath, or simple immersion of the hand in hot water. In a variation of these methods the trunk can be heated briefly with an electric lamp source without increasing core temperature. Normally, sweating will occur within 2 minutes without any patchiness, provided sweat glands are present. The sweating can be quantified by coating the area being tested with quinizarin powder, cobalt chloride solution, or iodine solution with starch, or by skin conductivity measurements or by using starch and iodine paper. If sweating does not occur, the significance of the finding is enhanced by demonstrating that sweat glands are actually present in the area tested. Pilocarpine (0.5 ml of 0.01% solution) intradermally stimulates sweat glands directly.

Skin vasodilation is tested using plethysmography to record hand blood flow. After exposure of the chest wall to radiant heat, skin vasodilation occurs, probably due to a reduction of sympathetic vasoconstrictor activity.

Cardiovascular System

The ANS has a strong influence on the cardiovascular system, controlling both heart rate and blood pressure. Sympathetic efferent fibers to capacitance and resistance vessels in muscles and the splanchnic area and to the kidneys, affecting renin release, represent the most important mechanisms preventing hypotension. Sympathetic and parasympathetic efferents control heart rate, the vagus altering the rate from beat to beat while the sympathetic provides a more gradual cardioaccelerator effect. Tests of the cardiovascular system can be divided into those testing parasympathetic function (response of the heart rate to deep breathing, the Valsalva maneuver, or standing) and others testing sympathetic function (response of blood pressure to standing or to sustained hand grip, stress tests). Tests of the aortic and carotid baroceptors can also be employed.

Normally the heart rate varies continually, increasing with inspiration and decreasing with expiration (sinus arrhythmia). The variation is abolished by atropine but uninfluenced by propranolol. However, isoproterenol also, to some extent, decreases the beat-to-beat variation in normal subjects. Therefore, heart rate variation is not solely dependent on parasympathetic activity, although commonly used for assessment of this limb of the ANS. With cardiovascular autonomic neuropathy, the variation in heart rate is reduced and even lost completely. The *heart rate variation* can be measured easily and provides the simplest and most sensitive index of autonomic damage.

The patient sits quietly and breathes deeply at 6 breaths a minute for 1 minute. An electrocardiogram (lead II) is recorded throughout the period of deep breathing, with a marker used to indicate the onset of each inspiration and expiration. The maximum heart rate during inspiration and the minimum heart rate during expiration is calculated for each breath, and the mean of the difference between maximum and minimum heart rate for the 6 breaths represents the result of the test. A variation of less than 10 beats per min is definitely abnormal, 11 to 14 is borderline, and 15 or more is a normal result.

During the *Valsalva maneuver* (after a deep inspiration, the patient performs a forced expiration against standardized resistance), blood pressure falls and heart rate increases; after the breath is released, the blood pressure rises and overshoots its original value, while the heart rate slows down. The changes in heart rate are mediated by the vagus nerve while the post-Valsalva hypertension can be prevented by sympathetic blockade.

The test is performed by the patient in the sitting or supine position blowing into a mouthpiece connected to a modified sphygmomanometer and holding the pressure at 40 mm Hg for 15 seconds while a continuous ECG (lead II) is recorded. The result is expressed as the Valsalva ratio, which is the ratio of the longest R–R interval after the maneuver to the shortest R–R interval during the maneuver. A ratio of 1.20 or more is considered normal.

During the *change from lying to standing*, a characteristic rapid increase in heart rate occurs, which is maximal at about the fifteenth beat after standing. This is followed by

an overshoot bradycardia, which is maximal at about the thirtieth beat after standing. The vagus mediates the changes in heart rate.

The patient changes from the lying to the standing position while a continuous ECG (lead II) is recorded. The result is expressed as the ratio between the R–R interval at beat 30 and the R–R interval at beat 15. A ratio of 1.05 or higher is considered normal.

On standing, hypotension is normally prevented by peripheral vasoconstriction mediated by sympathetic efferents. Therefore, with severe sympathetic damage, *postural hypotension* results.

The test is performed by measuring blood pressure with a sphygmomanometer while the patient is supine and then standing. A fall in systolic blood pressure of less than 10 mm Hg would be normally expected. In autonomic neuropathy, a fall of 20 mm Hg or more is found.

During *sustained hand grip,* a sharp rise in blood pressure occurs due to a heart rate dependent increase in cardiac output with unchanged peripheral resistance. With extensive sympathetic damage, the rise in blood pressure is abnormally small or nonexistent.

Using a hand grip dynamometer, a maximum voluntary contraction is first determined and then maintained at 30% of that maximum for as long as possible. The blood pressure is measured before and at 1-minute intervals during hand grip. The result is expressed as the difference between the highest diastolic pressure during hand grip and the diastolic pressure before hand grip. A rise of more than 10 mm Hg during hand grip is normally expected.

Stress tests, which are mediated by the sympathetic efferent pathway, cause vasoconstriction, hypertension, and tachycardia. They include sudden cortical arousal by unexpected noise, mental arithmetic, and the cold pressor test (hand immersed in water at 4°C for 90 seconds). These tests are not as well standardized as the ones described above.

The aortic and carotid baroceptors sense changes in blood pressure, and a sudden fall or rise in blood pressure is followed by a reflex increase or decrease in heart rate, predominantly through a vagal response. Blood pressure can be raised briefly by injecting phenylephrine intravenously. Amyl nitrite inhalation or nitroglycerine tablets can be used to cause a transient fall in blood pressure. These tests of the *baroreflex arc* are not used routinely because interpretation of individual results is difficult.

Ventilatory/Respiratory System

The role of the vagus nerve in the ventilatory/respiratory system is undoubted, and it is therefore not unexpected that abnormalities may occur in the patient with autonomic neuropathy, as outlined below. However, tests investigating these aspects of autonomic function are still, to a certain extent, of an experimental nature.

The response of carotid body chemoreceptors to *hypoxia* in autonomic neuropathy is controversial, although in animals vagal blockade or destruction of the carotid bodies abolishes the increased rate of breathing expected in response to hypoxemia.

On the other hand, *hyperventilation* reduces P_{CO_2}, which leads to cerebral vasoconstriction, muscle vasodilatation, and a fall in blood pressure if the vasomotor center is functioning normally. Reflex vasoconstriction and a return of the blood pressure to normal should follow if the vasomotor center is intact.

Bronchial airway tone is mainly under vagal control. Patients with autonomic neuropathy lose the bronchodilatory effect that should occur in response to local *cholinergic blockade* and do not show a fall in airway conductance after a provocation test with cold air.

During sleep, breathing is controlled by the medullary/respiratory centers and influenced by afferent input from central and peripheral receptors and the vagus nerves. Therefore, patients with autonomic neuropathy may have features of the sleep apnea syndrome, and apneic episodes have been demonstrated during both rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. In addition, the duration of REM sleep may be reduced.

Gastrointestinal System

The gastrointestinal tract is frequently involved by vagal autonomic neuropathy; the esophagus, stomach, small intestine, large intestine, and anal sphincter may all be affected. Abnormal patterns on *esophageal manometry* have been described following vagal damage. Slowed gastric emptying, in particular of the solid phase of a meal, may be investigated by *scintigraphic techniques* using a solid meal labeled with a radioactive marker and external scanning. *Barium studies* offer a less satisfactory alternative test. *Gastric acidity* is lost secondary to vagotomy; similarly, the vagally mediated gastric acid secretion response to insulin hypoglycemia can be impaired in patients with autonomic neuropathy. Increased *breath hydrogen* recorded after the ingestion of a nonabsorbable carbohydrate (lactulose) frequently confirms a prolonged transit time through the small intestine of patients with autonomic neuropathy. As a result, upper small bowel bacterial overgrowth can occur and increased *breath* CO_2 excretion following ingestion of an oral dose of labeled ^{14}C-glycoholic acid may provide evidence of bacterial bile acid deconjugation in patients with visceral neuropathy and diarrhea. Finally, *anal sphincter manometry* may reveal abnormal internal anal sphincter function in patients with incontinence secondary to autonomic neuropathy.

All the tests mentioned above have a place in the investigation of a patient with symptoms suggestive of gut involvement secondary to autonomic neuropathy, but the tests should be used selectively and are generally not used as screening tools for autonomic neuropathy.

Genitourinary System

Micturition follows activation of the parasympathetic pathways to the detrusor muscle and inhibition of the somatic input to the external urethral sphincter. The sympathetic system promotes urine storage by increasing urethral resistance and depressing detrusor contractions. Impairment of bladder function can be the result of different pathyphysiological events involving the peripheral autonomic nerves, the spinal cord, or suprasegmental autonomic centers. In diabetes, afferent fiber damage results in diminished bladder sensation, while damage to the parasympathetic innervation leads to decreased tone and weakness of the detrusor muscle. In contrast, loss of sympathetic innervation of the trigone and internal sphincter causes sphincter dysfunction.

Detailed *evaluation of bladder function and emptying* could include cystometry, sphincter electromyography, uroflow-

metry with measurement and recording of urinary flow, urethral pressure profiles, and electrophysiological tests of bladder wall innervation. A more limited study would include a sonogram to detect the presence of residual urine in the bladder and use of a flow meter to record the pattern, rate, and duration of urine flow. Dye studies (e.g., intravenous pyelogram) should only be used cautiously, keeping in mind the possible deterioration in renal function they can induce in patients with pre-existing renal disease. In the typical neurogenic bladder of diabetic patients, urodynamic studies show diminished awareness of filling, only a small rise in pressure with few or no contractions, increased bladder capacity, and on voiding a low flow rate with diminished detrusor activity.

Sexual dysfunction can be a feature of autonomic neuropathy in the male, parasympathetic disease leading to loss of erection and sympathetic damage to failure of ejaculation or retrograde ejaculation. Impotence is the norm among patients with bladder dysfunction but often occurs as an isolated problem. In the large diabetic population with impotence (30 to 50% of male diabetics), peripheral neuropathy is commonly present, while other tests of autonomic neuropathy (cardiovascular tests) may be normal. Although impotence may have a neurogenic etiology, vascular and psychogenic causes should also be considered. During dreaming sleep, characterized by REM periods, the normal male develops erections, and *nocturnal penile tumescence* can be monitored using a strain gauge loop around the penis. Nocturnal penile tumescence is reduced or absent in patients with organic impotence, while nocturnal erections of normal frequency and amplitude are found in psychogenic impotence.

The Pupil

The pupil has reciprocal innervation from the parasympathetic and sympathetic systems. The parasympathetic supply is concerned with constriction and the sympathetic with dilation. Long-term diabetics have abnormally small pupil diameters in the dark because of damage to the sympathetic innervation to the dilator muscles. The light reflex appears to be normal when pupil size is taken into account. Spontaneous oscillations in pupil size (hippus), which are observed normally, are also lost in autonomic neuropathy. The above pupil abnormalities correlate with abnormal cardiovascular tests indicative of autonomic neuropathy.

A number of tests have been described to investigate *pupillary responses* and to localize neurologic damage. When a solution of metacholine (similar effects to acetylcholine) is instilled in the eye, the normal pupil does not respond. If there is parasympathetic damage, the constrictor muscles are supersensitive and metacholine will cause pupillary constriction. Neither epinephrine nor phenylephrine causes any change in normal pupil size. However, if there is a sympathetic lesion, dilation occurs due to denervation hypersensitivity. With homatropine, the normal pupil becomes widely dilated, but in the patient with sympathetic damage, the dilation is slight or absent. Cocaine leads to dilation of the pupil by sympathetic activity, an effect that is absent when sympathetic damage occurs.

Endocrine System

Defects in *hormone secretion* in response to different stimuli appear to be a definite component of the autonomic failure syndrome. An impaired pancreatic polypeptide response to insulin hypoglycemia has been observed in vagotomized subjects and occurs in subjects with damage to the parasympathetic pathways. An impaired glucagon response to insulin hypoglycemia is probably also secondary to damage of the same pathways. Impaired release of catecholamines has also been demonstrated in diabetic patients with autonomic neuropathy and loss of warning symptoms (tremor, sweating, tachycardia) of hypoglycemia.

Due to *denervation hypersensitivity*, alpha-adrenergic agonists, such as norepinephrine, lead to accentuated vasoconstriction in patients with autonomic neuropathy. Similarly, the beta-adrenergic effects of epinephrine are enhanced and cause exaggerated hyperglycemia and vasodilatation with a drop in blood pressure.

Basic Science

Several conditions can impair autonomic function and the afferent, efferent, or central components of the ANS may be affected separately or together. These disorders can be categorized into: (1) systemic diseases (e.g., diabetes mellitus, alcoholism, amyloidosis); (2) primary neurologic diseases (e.g., tabes dorsalis and parkinsonism); (3) idiopathic degenerative diseases (e.g., progressive autonomic failure [PAF] without associated neurologic disorders, PAF associated with Parkinson's disease, PAF associated with multiple system atrophy [Shy–Drager syndrome]); and (4) miscellaneous causes (e.g., hypokalemia).

Of these conditions, autonomic derangement secondary to diabetes mellitus is by far the most common, 30 to 40% of patients with the disease having symptomatic or more commonly asymptomatic involvement as determined by clinical features and/or sensitive tests. The involvement of the autonomic nervous system may be patchy in distribution and is generally irreversible, although the symptoms may be curiously intermittent. Peripheral neuropathy is usually also present in patients with autonomic neuropathy. Symptomatic autonomic neuropathy in diabetics is associated with a bad prognosis, according to one estimate mortality reaching 50% within a 2.5-year period.

The particular autonomic functions affected differ from case to case according to the end organ involved. Although the site of the lesion in the reflex arc (afferent, central, or efferent) is of interest, it is frequently difficult to pinpoint and not always of practical value to identify. The tests most commonly performed to investigate autonomic function take into consideration the different organ systems comprising the ANS. They differ in complexity and at their simplest can be performed at the bedside.

Clinical Significance

Since there is no organ in the body without autonomic innervation, autonomic neuropathy represents a multisystem disease. As the pattern of involvement varies, investigations should be chosen judiciously from the multitude of tests described, taking into consideration the patient's presenting complaints. In contrast, cardiovascular tests lend themselves best for screening of large numbers of patients, and their interpretation is much easier than other tests because they have been thoroughly standardized. Therefore, in diabetic patients who are being screened for autonomic neuropathy

or in whom there are symptoms suggestive of autonomic neuropathy, the following tests would be most appropriate:

1. The heart rate response to the Valsalva maneuver, the heart rate variation on deep breathing, and the 30/15 heart rate ratio on standing, all of which, if abnormal, reveal evidence of parasympathetic damage.
2. Investigation for a postural drop in blood pressure and the blood pressure response to sustained hand grip; both tests, if abnormal, yield evidence of sympathetic damage.
3. The pancreatic polypeptide response to insulin hypoglycemia, which is impaired in patients with parasympathetic pathway damage.

In general, parasympathetic damage occurs earlier and is more prominent than sympathetic disease in diabetes. However, this finding may just reflect the greater sensitivity of tests available for assessment of parasympathetic activity. Diabetic patients with symptoms of genitourinary tract or gastrointestinal tract involvement should have the appropriate tests, as described in the previous sections, to confirm that indeed their clinical problems are secondary to ANS disease.

In progressive autonomic failure with postural hypotension, a sympathetic lesion is responsible. In an attempt to localize the lesion in the reflex arc (afferent, central, or efferent connections) a number of tests may be performed:

1. If the vasoconstrictive response to stress is preserved, an afferent or central lesion is likely.
2. If the sweating response to a rise in body temperature is defective, the lesion is probably efferent.
3. Normal piloerection and sweating to intradermal acetylcholine indicates a preganglionic lesion, whereas a defective response indicates a postganglionic lesion.
4. Following postganglionic denervation there is supersensitivity of the vessels to the transmitter norepinephrine and a loss of response to tyramine.

These tests usually provide evidence of an efferent sympathetic lesion that is more preganglionic in progressive autonomic failure with multiple system atrophy (Shy–Drager syndrome) and more postganglionic in progressive autonomic failure alone.

When investigating the parasympathetic/sympathetic innervation of the pupil it is best to proceed as follows:

1. When the pupil is dilated, if constriction occurs with metacholine, a parasympathetic lesion is present; if there is no change, a sympathetic lesion is responsible.
2. When the pupil is small, if dilation occurs with homatropine, there has been parasympathetic overactivity; if only slight enlargement occurs, a sympathetic lesion is likely.
3. In the latter case, with a small pupil and a sympathetic lesion, cocaine is tried next. If dilation occurs, the lesion is in the CNS; if no dilation occurs, the lesion may be outside the CNS.

The best example of a parasympathetic lesion of the pupil is provided by the Holmes–Adie pupil, a dilated pupil with slow constriction on prolonged light stimulation. Horner's syndrome is the result of interruption of the sympathetic pathways, and a small pupil is part of the wider clinical picture of sympathetic damage that includes ipsilateral miosis, ptosis, enophthalmos, and loss of sweating on the same side of the face.

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